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(54) EXTRACT OF AMBELIFERAE FOR PHARMACOLOGICAL USE

(71) I, ATHANASIOS ALEXANDROU SKOURIDES, of 41 Devonshire Road, Mill Hill, London, NW7 1NE, of British Nationality, do declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a preparation having therapeutic anti-inflammatory and anti-coagulant activity and having particular utility in the treatment of rheumatism and allied disorders. It is also believed that the same or similar active principles are present in other plants of the ambeliferae family. According to the present invention there is provided a process for the production of a pharmacologically active substance (having antiinflammatory and anticoagulant activity) which comprises extracting the tuberous portion of a plant of the ambeliferae family. An example of such plants is *Ferula Communis*. The tubers or rhizomes are preferably crushed and the juices squeezed from the crushed materials. A solvent may be used to extract the active substance and normally water is used but other solvents can be employed including chloroform or ether.

Increased activity is noted when an aqueous extract of the plant is subjected to continuous ether extraction and the continuous extraction may be carried out on the original plant.

It is desirable to add a preservative to the extracted substance (for example ether or alcohol) and suitable flavouring materials may be added to the substance in order to make it more palatable, for example fruit juices such as orange or grape juice.

Alternative procedures for extracting the substance of the invention:—

1. By crushing the fresh tubers, expressing and concentrating the juice obtained and adding a preservative as above or nipa sept.

2. By drying the tubers and crushing and comminuting and extracting the substance of the invention with a suitable solvent and subsequently encapsulating or compounding in any conventional pharmaceutically accepted diluents or carriers.

The following example is given to illustrate the method of extracting the active substance of the invention from the plant *Ferula Communis*.

Example.

The tuberous roots of the plant *Ferula Communis* were thoroughly washed and peeled and 200 drams of the flesh of the tubers were cut up into small pieces, added to 400 drams of water and boiled until entirely soft. After squeezing thoroughly the flesh of the tubers under the surface of the water, the aqueous extract was filtered off and evaporated to remove excess water. When the aqueous extract had been concentrated to a pasty gelatinous mass, ethyl alcohol was added as a preservative in order to ensure protection against growth of micro-organisms. Fruit juices such as orange and grape juice may be added to the substance in order to render it more palatable and the resultant preparation may be utilised in this form for the treatment of human rheumatism. The resultant preparation was sufficient for three doses.

In a modification of the extraction procedure the pasty gelatinous mass obtained by concentration of the aqueous extract may be subjected to continuous ether extraction followed by evaporation of the ether to produce a gum. The resultant gum shows a higher activity than the aqueous extract and can be dissolved in dimethyl formamide for administration.

The preparation may, of course, be encapsulated or compounded in any conven-

tional pharmaceutical formulation using pharmaceutically acceptable diluents or carriers.

The following pharmacological tests demonstrate the activity of the preparation in accordance with the invention. For the purpose of the pharmacological tests the active substance was prepared in accordance with the modified procedure described above and was administered to the laboratory animals as a solution in dimethyl formalide emulsified in water using "Tween 30" so ("Tween" is a Registered Trade Mark) that the final emulsion contained no more than 5% dimethyl formalide. The tests carried out and the results are given in the following description.

A. Carrageenin Oedema of the Rat Foot.

The carrageenin oedema test of Winter et al Proc. Soc. Exptl. Biol. Med. III, 544, (1962) was used.

Groups of four rats were dosed orally with the test materials at $\frac{1}{2}$ hour and 3 hours prior to the injection of 0.1 ml. of a 1.0% suspension of carrageenin into the plantar surface of the right hind foot of each rat. Injected foot volumes were measured initially and $2\frac{1}{2}$ hours after the carrageenin. The mean increase in foot volume for each group was calculated and the results expressed as a percentage inhibition of swelling compared to a control group of ten animals, dosed with saline and tested concurrently.

In one experiment, the test was carried out on animals which had been bilaterally adrenalectomised one week previously, in order to establish whether the anti-inflammatory activity was mediated through the release of adrenal hormones.

The results are given in Table 1 and clearly indicates that the substance prepared in accordance with the invention has pronounced anti-inflammatory activity and that the activity is not affected by the presence of adrenal hormones.

B. Ultra-Violet Induced Erythema in Guinea-Pigs.

The backs of groups of four guinea pigs were clipped and depilated with 'Veet O', (Trade Mark) proprietary depilatory cream. The test materials were given orally and 1 one later, four sites on the back of each guinea pig were irradiated for 60 seconds with a Kromayer Lamp (Kromayer Lamp is a Registered Trade Mark). The degree of erythema produced was assessed $2\frac{1}{2}$ hours after irradiation using an arbitrary scoring system and the results expressed as a percentage reduction in erythema, compared to a control group, treated similarly, but dosed with saline.

The results (Table 2) show that significant activity was apparent on this test and also the activity was dose related.

C. Anti-Coagulant Activity.

i. Whole Blood Clotting Time.

Clotting times were carried out using the simple capillary tube method.

Glass tubes, 120 mm. long and 1 mm. bore were scored at 5—10 mm. intervals with a diamond. Blood was drawn into the tubes by capillary action from the cut ends of the tails in rats and from the extraorbital venous plexus in mice lightly anaesthetised with ether. A clock was started when a large drop had been drawn into the tube and taken to be time zero. After the tube had been filled the tubes were carefully broken at the score marks at about 15 second intervals until a threadlike clot appeared between the broken ends of the tube and the time was noted. This was taken to be the clotting time.

Experiments were carried out in order to determine whether the substance of the present invention was active in both rats and mice and comparisons were made with dicoumarol in mice.

Table 3 shows the results obtained in rats and mice. It can be seen that increased blood clotting times occurred in both rats and mice. In rats blood clotting times had returned to normal, 8 days after treatment stopped. In mice, the effect was dose-related, and appeared to be maximal 48 hours after the last dose.

ii. Plasma Prothrombin Estimation.

Prothrombin times were measured using a modification of Quick's method (J. Amer. Med. Ass. 110, 1658 (1938)) in which a commercial preparation of thromboplastin (Thrombokinase "Geigy" with calcium) was used. 0.2 ml. of homogeneous suspension of thromboplastin containing excess calcium, prepared from the tablets supplied, was added to 0.1 ml. of citrated plasma at 37° C and immediately mixed. The time from mixing to coagulation was measured and was taken to be the prothrombin time. Coagulation was assessed by removing the tubes from the water bath at frequent intervals for examination.

Prothrombin estimations were made on rats pretreated with the substance of the invention or dicoumarol and the results (Table 4) show that both drugs caused increased prothrombin times. The effect with the substance of the invention was much more marked than with dicoumarol.

5 In a different concentration the substance of the invention might be effective as a rodenticide. 5

TABLE 1

EFFECT OF SUBSTANCE OF INVENTION ON CARRAGEENIN OEDEMA IN RATS

Dose (mls./kg.)	Reduction of Oedema (percent)	Test Number
2 × 10	32.7	1
2 × 10	17.5	2
		3
		Sample 1
2 × 10	38.4	4
2 × 10	23.0	5
		Sample 2
2 × 10	31.3	6
		Sample 3
2 × 10	65.0	8
2 × 10	63.5	9
		Sample 1

Tests 1 to 8 were carried out on normal rats while test 9 was carried out on adrenalectanised rats.

TABLE 2

Dose mls./kg.	Percent Reduction of Erythema	Remarks
10	25	Sample 1
a		
10	33.4	Sample 2
20	50.0	

EFFECT OF SUBSTANCE OF INVENTION ON U.V. ERYTHEMA IN GUINEA PIGS

TABLE 3
EFFECT OF SUBSTANCE OF INVENTION ON WHOLE BLOOD CLOTTING TIMES IN RATS AND MICE

Species	Treatment with substance Dose (mls./kg.)	No. of daily doses.	Time of test after last dose	No. of Animals	Mean Clotting Time (Secs.)	Remarks
Rat	Nil	—	—	5	87	Normal rats
Rat	10	4	48 hours	5	150	„ „
Rat	Nil	—	—	5	53	Normal rats
Rat	10	4	8 days	5	63	„ „
Mouse	Nil	—	—	10	58	—
Mouse	5	2	48 hours	5	106	—
Mouse	10	2	48 hours	5	120	—
Mouse	20	2	48 hours	5	205	—
Mouse	Nil	—	—	10	63	—
Mouse	5	2	24 hours	5	115	—
Mouse	10	2	24 hours	5	102	—
Mouse	20	2	24 hours	5	157	—

TABLE 4

EFFECT OF SUBSTANCE OF INVENTION AND DICOUMAROL ON PROTHROMBIN TIMES IN RATS

Treatment with anti-coagulant			Time of Test after last dose	No. of Animals	Mean pro- thrombin time (Secs)
Compound	Dose	No. of doses (in 24 hrs.)			
Control	—	—	—	5	13.9
Substance of invention	10 mls./kg	3	24 hours	5	318.0
Control	—	—	—	4	13.0
Dicoumarol	50 mg./kg.	2	24 hours	4	76.0

The results of the pharmacological tests on the substance of the present invention show that it possesses anti-inflammatory activity which is not mediated through the adrenal glands. The effect is not due to a diuretic action as the preparation showed no increase diuresis in water-loaded rats, compared with an untreated control group.

The substance of the present invention has significant anti-coagulant activity which appears to be of the Dicoumarol type. It is, however, relatively more active than dicoumarol in increasing prothrombin time. Tests have also shown that the material does not appear to contain either coumarine or indane diones.

WHAT I CLAIM IS:—

1. A process for the production of a pharmacologically active substance, having anti-inflammatory and anti-coagulant activity, which comprises extracting the tuberous portion of a plant of the ambeliferæ family.

2. A process according to claim 1 in which the plant is *Ferula Communis*.

3. A process according to claim 1 or claim 2 in which the tubers or rhizomes are crushed and the juices squeezed from the crushed materials.

4. A process according to any one of the preceding claims in which the tubers or rhizomes are treated with a solvent to extract the pharmacologically active substance.

5. A process according to claim 4 in which the solvent is water, ether or chloroform.

6. A process for the preparation of a pharmacologically active substance substantially as described with reference to the Example herein.

7. A pharmaceutical preparation effective in the treatment of rheumatism or gout which comprises an active substance obtained by the process claimed in any one of the preceding claims.

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